

Multidrug resistant bacteria isolated from septic arthritis in horses¹

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ABSTRACT- Motta R.G., Martins L.S.A., Motta I.G., Guerra S.T., De Paula C.L., Bolanos C.A.D., Silva R.C. & Ribeiro M.G. 2017. **Multidrug resistant bacteria isolated from septic arthritis in horses.** *Pesquisa Veterinária Brasileira* 37(4):325-330. Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Cx. Postal 560, Botucatu, SP 18618-681, Brazil. E-mail: rgmotta@fmvz@gmail.com

Septic arthritis is a debilitating joint infectious disease of equines that requires early diagnosis and immediate therapeutic intervention to prevent degenerative effects on the articular cartilage, as well as loss of athletic ability and work performance of the animals. Few studies have investigated the etiological complexity of this disease, as well as multidrug resistance of isolates. In this study, 60 horses with arthritis had synovial fluid samples aseptically collected, and tested by microbiological culture and *in vitro* susceptibility test (disk diffusion) using nine antimicrobials belonging to six different pharmacological groups. Bacteria were isolated in 45 (75.0%) samples, as follows: *Streptococcus equi* subsp. *equi* (11=18.3%), *Escherichia coli* (9=15.0%), *Staphylococcus aureus* (6=10.0%), *Streptococcus equi* subsp. *zooepidemicus* (5=8.3%), *Staphylococcus intermedius* (2=3.3%), *Proteus vulgaris* (2=3.3%), *Trueperella pyogenes* (2=3.3%), *Pseudomonas aeruginosa* (2=3.3%), *Klebsiella pneumoniae* (1=1.7%), *Rhodococcus equi* (1=1.7%), *Staphylococcus epidermidis* (1=1.7%), *Klebsiella oxytoca* (1=1.7%), *Nocardia asteroides* (1=1.7%), and *Enterobacter cloacae* (1=1.7%). Ceftiofur was the most effective drug (>70% efficacy) against the pathogens in the disk diffusion test. In contrast, high resistance rate (>70% resistance) was observed to penicillin (42.2%), enrofloxacin (33.3%), and amikacin (31.2%). Eleven (24.4%) isolates were resistant to three or more different pharmacological groups and were considered multidrug resistant strains. The present study emphasizes the etiological complexity of equine septic arthritis, and highlights the need to institute treatment based on the *in vitro* susceptibility pattern, due to the multidrug resistance of isolates. According to the available literature, this is the first report in Brazil on the investigation of the etiology of the septic arthritis in a great number of horses associated with multidrug resistance of the isolates.

INDEX TERMS: Joint infectious diseases, horses, etiology, antimicrobial resistance, arthritis.

RESUMO.- [Bactérias multirresistentes isoladas de artrite séptica equina.] Artrite séptica é uma artropatia infecciosa debilitante de equinos, que requer diagnóstico

precoce e intervenção terapêutica imediata, com intuito de evitar a degeneração de a cartilagem articular e a perda da capacidade atlética e de trabalho dos animais. Poucos estu-

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dos têm investigado a complexidade etiológica da afecção, bem como a presença de multirresistência dos isolados aos antimicrobianos. Foram investigados 60 equinos portadores de artrite, submetidos à colheita asséptica de líquido sinovial para a realização de cultivo microbiológico e teste de sensibilidade microbiana *in vitro* (difusão com discos) com nove antimicrobianos pertencentes a seis diferentes grupos farmacológicos. Foi obtido isolamento microbiano em 45 (75,0%) amostras, como segue: *Streptococcus equi* subsp. *equi* (11=18,3%), *Escherichia coli* (9=15,0%), *Staphylococcus aureus* (6=10,0%), *Streptococcus zooepidemicus* (5=8,3%), *Staphylococcus intermedius* (2=3,3%), *Proteus vulgaris* (2=3,3%), *Trueperella pyogenes* (2=3,3%), *Pseudomonas aeruginosa* (2=3,3%), *Klebsiella pneumoniae* (1=1,7%), *Rhodococcus equi* (1=1,7%), *Staphylococcus epidermidis* (1=1,7%), *Klebsiella oxytoca* (1=1,7%), *Nocardia asteroides* (1=1,7%) e *Enterobacter cloacae* (1=1,7%). Cefotiofur foi o antimicrobiano mais efetivo (>70% eficácia) *in vitro* diante dos patógenos. Em contraste, alta resistência dos isolados (>70% de resistência) foi observada para penicilina (42,2%), enrofloxacin (33,3%) e ampicilina (31,2%). Onze (24,4%) isolados foram resistentes a três ou mais diferentes grupos de fármacos e considerados com resistência múltipla aos antimicrobianos. O presente estudo enaltece a complexidade etiológica envolvida na artrite séptica em equinos e ressalta a necessidade de instituir o tratamento dos animais com respaldo de testes de sensibilidade microbiana *in vitro* em virtude da resistência múltipla dos isolados. De acordo com a literatura consultada, esta é a primeira descrição no país da etiologia da artrite séptica em grande número de equinos associada a multirresistência dos isolados aos fármacos testados.

TERMOS DE INDEXAÇÃO: Artrite infecciosa, cavalos, etiologia, multirresistência aos antimicrobianos, artrite.

INTRODUCTION

Equines are in contact with humans for thousands years, being used for transport, work, leisure, food, entertainment, sports and, more recently, to recovery of adults and children with cognitive or psychomotor problems (Thomassian 2005). Brazil has the fourth largest herd of equines of the world, with about 8,000,000 animals, and it only behind of China, Mexico, and EU. Horse breeding and raising generate 3.2 million direct and indirect jobs, moving US\$7.3 billion with equine rearing, and US\$4.4 million with the exports of animals. In addition, Brazil ranks eighth in worldwide horse meat exports, selling the product to Belgium, the Netherlands, Italy, Japan, France, and the United States (Brasil 2016).

Joint diseases are problematic conditions for horses and can lead to permanent lameness, functional incapacity, or death due to septic shock in severe cases (Beccati et al. 2015). Septic arthritis is an inflammatory process of the joints caused by bacteria, fungi, and viruses (Taylor et al. 2010). It is possible that all joint structures are affected, which may cause clinical manifestations according to the location of the lesion (Bertone 2003, Olive et al. 2014). Septic arthritis is usually caused by bacteria. Pathogens reach the joint via blood, local invasion (traumatic inoculation),

or by iatrogenic route, a consequence of local contamination after direct infiltration of a drug into the joint (Colahan et al. 2000, Hall et al. 2012).

The clinical signs of the septic arthritis in equines include lameness of variable intensity, edema, local hyperthermia, effusion, and marked sensitivity to pain at palpation (Thomassian 2005, Steel et al. 2013). In its initial course, diagnosis is based on clinical signs, rigorous clinical examination of the affected joints and limbs, and identification of potential risk factors in the anamnesis (recent traumas, intra-articular injections, systemic diseases, and immunosuppression) (Olive et al. 2014). Diagnosis is confirmed by arthrocentesis, followed by laboratory analysis of the synovial fluid, microbial culture, *in vitro* susceptibility test, and imaging tests of the affected joint (Taylor et al. 2010, Hall et al. 2012). In septic arthritis, the synovial fluid can be turbid, with purulent or hemorrhagic aspect, and may present altered viscosity. The liquid shows increased inflammatory cell counts and protein concentration (Haerdi-Landerer et al. 2010). Early diagnosis of the causative agent is necessary, because many animals evolve rapidly to chronic and debilitating lesions, and the animal may not support its weight on the affected limb. Consequently, the animal will gradually lose joint function, tending to decubitus and leading to a poor prognosis (Forresu et al. 2006, Olive et al. 2014).

Treatment focuses on the use of antimicrobial drugs and articular drainage, and aims at the elimination of the causative agent, and removal of cell debris and fibrin from the joint, which may damage the articular cartilage (Haerdi-Landerer et al. 2010). Microbial culture, associated with the *in vitro* susceptibility tests, increases therapy efficiency (Morton 2005, Taylor et al. 2010).

Different antimicrobial drugs are used to treat septic arthritis, as monotherapy or in association, via intra-articular and/or parenteral injection (Haerdi-Landerer et al. 2010, Beccati et al. 2015), including β -lactams, aminoglycosides, sulfonamides, fluoroquinolones, macrolides, rapamycin, and amphenicols (Morton 2005, Giguère et al. 2010, Hall et al. 2012). Broad spectrum antimicrobials and those that reach high intra-articular concentrations should be preferred (Schneider et al. 2002, Haerdi-Landerer et al. 2010).

The multidrug resistance of bacterial species is an emergent global concern included in the "One Health" concept (Ribeiro et al. 2015), and poses a major threat to control and treatment strategies of many infectious diseases that affect domestic animals (Giguère et al. 2010). Nevertheless, to date, the prevalence of drug resistance or the degree of multidrug resistance of pathogens isolated from septic equine arthritis is unclear or unnoticed.

Despite the severity of the clinical cases of septic arthritis, few comprehensive studies have investigated the etiologic complexity of the disease (Schneider et al. 2002, Bertone 2003, Morton 2005, Carstanjen et al. 2010), as well as the occurrence of multidrug resistance to the most common antimicrobials used in the treatment of the disease. In Brazil, no systemized study has been focused on the main pathogens causing septic arthritis, in large number of equines, or on the investigation of multidrug resistance of the isolates. Thus, the present study aimed to investigate the microbial

etiology of septic arthritis in 60 horses, as well as the occurrence of multidrug resistance of the isolates to the main antimicrobials used to treat the disease in this species.

MATERIAL AND METHODS

Sixty horses with septic arthritis, 28 (46.7%) males and 32 (53.3%) females, were studied from 2013 to 2015. The animals showed the following distribution according to breed: Quarter Mile Horse (n=17; 28.3%), Mangalarga Marchador (n=10; 16.7%), Criollo (n=6; 10%), Appaloosa (n=3; 5%), Paint Horse (n=8; 13.3%), and mixed breed horses (n=16; 26.7%). Age ranged from 2 to 11 years, with 20 (33.3%) animals between 2-3 years; 31 (51.7%) between 4-5 years; 3 (5.0%) animals between 6-7 years, 2 (3.3%) animals between 8-9 years and 4 (6.7%) >10 years. Animals were used for tie-down roping (n=16; 26.7%), riding (n=19; 31.7%), barrel racing (n=14, 23.3%), and marching (n=11; 18.3%), and came from different farms of Rio Verde, GO, Brazil (17°47'53"S, 50°55'41"W), and its surrounding areas.

Horses with lameness (variable intensity), increased articular volume, occasionally effusion in severe or chronic cases, hyperthermia, joint enlargement, and/or marked local pain sensibility were considered highly suggestive of having septic arthritis. Once signs were compatible with the disease, local antiseptics of the affected joint(s) was performed with 2% povidone iodine solution, and 1mL synovial fluid was aseptically drawn using a 5-mL disposable syringe and 30x7 disposable needles. The following joints were sampled: carpal (n=22; 36.7%), tarsal (n=16; 26.7%), femoro-tibio-patellar (n=11; 18.3%), proximal interphalangeal (n=5; 8.3%), metacarpal/metatarsal-phalangeal (n=4; 6.7%), and distal interphalangeal (n=2; 3.3%). Samples were kept at 4°C into sterile flasks and sent to the Veterinary Microbiology Lab, University of Rio Verde, GO, for microbiological culture, and *in vitro* susceptibility testing. No antimicrobials were used in horses sampled.

All the samples were plated simultaneously in defibrinated sheep blood agar (5%) and MacConkey agar, and incubated aerobically at 37°C for 72 hours. Isolated microorganisms were identified based on conventional phenotypic methods (Songer & Post 2005, Quinn et al. 2011). All isolates were subjected to an *in vitro* antimicrobial diffusion disk test, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2014), previously the National Committee for Clinical Laboratory Standards (NCCLS). Nine commercially available antimicrobials, used in livestock veterinary practice and/or indicated for equine septic arthritis were tested, totaling six different antimicrobials groups, as follows: β -lactam (ceftiofur, 30 μ g; penicillin, 10 IU), aminoglycosides (amikacin, 30 μ g; gentamicin, 10 μ g), analog of thiamphenicol (florphenicol, 30 μ g), fluoroquinolones (enrofloxacin, 5 μ g; norfloxacin, 10 μ g), sulfonamides (trimethoprim-sulfamethoxazole, 1.25/23.7 μ g), and macrolides (azithromycin, 15 μ g). Multidrug resistance was considered when one isolate showed simultaneous resistance to three or more antimicrobials (Schwarz et al. 2010, Girardini et al. 2013).

Considering a population of 11,000 equines in the studied region, and prevalence of septic arthritis ranging from 1 to 2%, the minimum number of animals with clinical signs to be sampled was estimated in 47 horses. Associations between the frequency of isolated microorganisms and the variables (anatomic location of the joints, age, breed, gender, and use of the animals) were tested using the Chi-square and/or Fisher exact test, considering $P < 0.05$ as significant (Triola 2005). The present study was approved by the Committee of Ethics and Animal Experimentation of University of Rio Verde (UniRV), GO, Brazil (Protocol #01/2015).

RESULTS

Bacteria were isolated in 45/60 (75%) synovial fluid samples from horses with arthritis. The most prevalent microorganisms were streptococci and enterobacteria, followed by staphylococci, order Actinomycetales, and *Pseudomonas aeruginosa* (Table 1).

Table 1. Groups and microorganisms isolated from 60 samples of synovial fluid from horses with septic arthritis. Rio Verde, GO, Brazil, 2013-2015

Groups	Microorganisms	Absolute frequency	Relative frequency (%)
Streptococci	<i>Streptococcus equi</i>	11	18.3
	<i>Streptococcus zooepidemicus</i>	5	8.3
	Subtotal = 16		26.6
Staphylococci	<i>Staphylococcus aureus</i>	6	10.0
	<i>Staphylococcus intermedius</i>	2	3.3
	<i>Staphylococcus epidermidis</i>	1	1.7
	Subtotal = 9		15.0
Enterobacteria	<i>Escherichia coli</i>	9	15.0
	<i>Proteus vulgaris</i>	2	3.3
	<i>Klebsiella pneumoniae</i>	1	1.7
	<i>Klebsiella oxytoca</i>	1	1.7
	<i>Enterobacter cloacae</i>	1	1.7
	Subtotal = 14		23.4
Order Actinomycetales	<i>Trueperella pyogenes</i>	2	3.3
	<i>Nocardia asteroides</i>	1	1.7
	<i>Rhodococcus equi</i>	1	1.7
	Subtotal = 4		6.7
Miscellaneous Gram-negative rods (non-enterobacteria)	<i>Pseudomonas aeruginosa</i>	2	3.3
	Subtotal = 2		3.3
Negative	-	15	25.0
Total	-	60	100.0

Streptococcus equi = *Streptococcus equi* subsp. *equi*, *Streptococcus zooepidemicus* = *Streptococcus equi* subsp. *zooepidemicus*.

The results on the most relevant antimicrobial susceptibility patterns for the isolates are shown in Table 2. The most effective drug (>70% efficacy) was ceftiofur (77.8%). High resistance rates (>30% inefficacy) were observed for penicillin (35.6%), enrofloxacin (33.3%), and amikacin (31.2%) (Table 2). Simultaneous drug resistance to three or more groups of drugs was observed in 11 (24.4%) isolates.

No significant association ($P>0.05$) was observed between the frequency of isolated microorganisms and the anatomical location of the sampled joints, age, gender, breed, and use of the animals.

Table 2. In vitro antimicrobial susceptibility profile (disk diffusion method) of microorganisms from equines with septic arthritis. Rio Verde, GO, Brazil, 2013-2015

Antimicrobials	Number of isolates (%)		
	Susceptible	Intermediate	Resistant
Ceftiofur	35/45 (77.8%)	5/45 (11.1%)	5/45 (11.1%)
Florfenicol	27/45 (60.0%)	12/45 (26.6%)	6/45 (13.4%)
Gentamicin	27/45 (60.0%)	11/45 (24.4%)	7/45 (15.6%)
Norfloroxacin	30/45 (66.7%)	10/45 (22.2%)	5/45 (11.1%)
Enrofloxacin	16/45 (35.6%)	14/45 (31.1%)	15/45 (33.3%)
Amikacin	20/45 (44.5%)	11/45 (24.4%)	14/45 (31.1%)
Penicillin	16/45 (35.6%)	10/45 (22.2%)	19/45 (42.2%)
Trimethoprim-sulfa	21/45 (46.6%)	17/45 (37.8%)	7/45 (15.6%)
Azithromycin	27/45 (60.0%)	8/45 (17.8%)	10/45 (22.2%)

Trimethoprim-sulfa = Trimethoprim-sulfamethoxazole.

DISCUSSION

We highlight in this study the complexity of groups and pathogens identified in septic arthritis of horses, as well as the multidrug resistance of the isolates to conventional antimicrobials recommended for the treatment of infectious diseases of the joints.

Streptococcus equi subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus* were the most prevalent bacterial group observed in this study. This group can be found in the skin, conjunctival and mucous membranes (mainly upper respiratory tract) of the animals, causing opportunistic infections in different organs and tissues (Songer & Post 2005). Particularly in horses, these pathogens are linked to the occurrence of strangles, a highly prevalent disease of the upper respiratory tract (Waller et al. 2014). The wide occurrence of these pathogens causing septic arthritis in horses can be justified by the presence of this group of microorganisms on the skin of animals, making it easy for traumatic inoculation into the articular region, contamination of intra-articular administration of drugs (iatrogenic route) (Steel et al. 2013), or systemic dissemination from the upper respiratory tract (Songer & Post 2005, Orsini 2006). In addition, Pelkonen et al. (2013) warned about the zoonotic potential of *Streptococcus equi* subsp. *zooepidemicus* in Finland as a causative agent of an emerging disease with severe evolution in humans, given the risk of transmission to humans that are in close contact with infected horses.

Staphylococci were also isolated from many animals in this study. Similar to the streptococci group, these microorganisms are found on the skin, conjunctival and mucous membranes, and may cause opportunistic infections in the joint by traumatic inoculation, iatrogenic or systemic

routes (Benites et al. 2016). Moreover, Taylor et al. (2010) referred to the refractoriness to treatment of equine septic arthritis caused by *Staphylococcus aureus*, as well as its poor prognosis, in Great Britain.

Enterobacteria presented high rates of isolation in the sampled horses. This complex group belongs to enteric microbiota of animals. These bacteria are also found in the environment, feces, water, organic material, or contaminated materials for general use in animal raising (Songer & Post 2005). They are responsible for enteric and extra-enteric opportunistic infections in animals, and have a wide and diverse set of virulence factors (Quinn et al. 2011). The high occurrence of enterobacteria in the synovial fluid samples of horses is in agreement with similar studies in other countries (Schneider et al. 2002, Bertone 2003), and probably is a consequence of traumatic contamination of the joint by environmental microorganisms, as well as being secondary to systemic dissemination (Steel et al. 2013).

Pseudomonas aeruginosa is a Gram-negative bacterium that affects humans and animals, and usually causes waterborne disease. It is found in the environment, intestinal tract of animals, or contaminated surgical materials. It causes opportunistic disease, and leads to many clinical signs in livestock (Songer & Post 2005, Quinn et al. 2011). In this study, *Pseudomonas aeruginosa* has been isolated in two animals with septic arthritis, probably secondary to contamination with water, needles during intra-articular inoculation of drugs, or feces from the environment (Steel et al. 2013). In these situations, prognosis is usually poor due to the high refractoriness of the pathogen to the conventional antimicrobials used to treat the disease (Songer & Post 2005, Quinn et al. 2011).

The order Actinomycetales is a heterogeneous group of microorganisms relevant to animal and human health, once they are related to high-impact livestock diseases (i.e. tuberculosis, rhodococcosis, nocardiosis, corynebacteriosis, and dermatophilosis), besides the risks these pathogens pose to public health (Songer & Post 2005). *Nocardia asteroides* and *Trueperella pyogenes* are aerobic actinomycetes related to opportunistic pyogranulomatous infectious in livestock (Quinn et al. 2011). Different species of *Nocardia* are found on the ground, particularly in sites where soil, organic material, and high humidity are found. *Trueperella pyogenes* is found on the skin and mucous membranes of the animals, and is usually transmitted by flies (Ribeiro et al. 2015). Although the occasional occurrence of this microorganism in horses, as observed in this study, lesions caused by these pathogens are intensely pyogenic, of difficult tissue repair, and usually refractory to the treatment with conventional antimicrobials, which frequently leads to chronic processes with poor prognosis (Quinn et al. 2011).

Rhodococcus equi is the main cause of morbidity and mortality by pneumonia in foals up to 6 months. In foals, the bacterium causes infectious or immune-mediated joint diseases. In immune-mediated conditions, the disease is secondary to the deposition of immune complexes on the joint, and it is not possible to isolate the bacterium from the synovial fluid. Here, *Rhodococcus equi* was detected in only one equine with septic arthritis, probably following a dis-

seminated infection from the lung or intestines. However, rhodococcosis is rare in adult horses, and usually occurs in weak animals or those co-infected with immunosuppressive agents (Ribeiro & Vargas 2016).

From 60 sampled horses, 15 (25 %) presented negative results in microbiological culture. In spite of the criteria used in the sampling collection, equine joint diseases may also be caused by traumatic, generative or immune-mediated processes (Thomassian 2005, Madigan & House 2006). In addition, some uncommon microorganisms in horses may occasionally cause joint, ligament, and bursa lesions secondary to systemic infections, i.e., *Brucella abortus* and *Mycoplasma* sp. These microorganisms need selective media or optimal oxygen tension conditions to be isolated, which were not used in the present study. Furthermore, according to what was mentioned above, in immune-mediated conditions caused by *Rhodococcus equi*, the articular lesion is caused by the deposition of immune complexes (antigen-antibody) and is not possible the isolation of the microorganism (Ribeiro & Vargas 2016). All these factors may justify, in part, the negative isolation of the microorganisms in 15 samples of synovial fluid.

No statistical association was observed between the isolated pathogens and age, breed, gender, use of the animals, and anatomical location of the sampled joints. These results agree with similar studies from other countries, in which no influence from the same variables were observed in septic joint diseases in horses (Carstanjen et al. 2010, Steel et al. 2013). However, in the current study, greater occurrence of equine septic arthritis was observed in Quarter Miles, Mangalarga Marchador, Paint Horse, and mixed breeds ranging from 2-5 years, with lesions on tarsal, carpal, and femoro-tibio-patellar joints. The bias of septic arthropathies in these breeds and age can be attributed to the use of these animals in the studied region, particularly for entertainment and sport (tie-down roping, riding, barrel racing, and marching); which require intense use, exposing and predisposing the animals to tarsal and carpal lesions, as well as lesions in patella and fetlock.

β -lactams (penicillin, cephalosporins), aminoglycosides (Morton 2005), and fluoroquinolones (Haerdi-Landerer et al. 2010) are the antimicrobials of choice for intra-articular and/or systemic therapy of equine septic arthritis. In this study, only ceftiofur revealed *in vitro* sensitivity >70% to all isolates. The high efficacy of ceftiofur may be credited to the fact that intensive use of this cephalosporin in equine practice therapy in Brazil took place only in the last decade (Andrade & Giuffrida 2008), reducing the selection pressure for multidrug resistance (Giguère et al. 2010). In contrast, high resistance rates (>30%) were registered for penicillin (42.2%), enrofloxacin (33.3%), and amikacin (31.2%). Interestingly, penicillin, enrofloxacin, and amikacin are the antimicrobials of choice in the treatment of equine septic arthritis (Morton 2005, Haerdi-Landerer et al. 2010). In addition, 11 (24.4%) isolates presented simultaneous resistance to three or more groups of antimicrobials tested, which is a phenotypic evidence for the presence of isolates harboring genes related to multidrug resistance (Schwarz et al. 2010).

The inappropriate or empiric use of antimicrobials increases the selection rate for multidrug resistant bacteria, which is an emergent global threat (Giguère et al. 2010). Based on these findings, the selection of first-choice antimicrobial therapy should be based on regional *in vitro* resistance profile. In fact, the responsible use of antimicrobials for animals is an emergent "One Health" concern (Ribeiro et al. 2015) in order to preserve these drugs for human therapy approaches.

CONCLUSIONS

This study highlights the complexity of bacterial agents involved in equine septic arthritis, with predominance of streptococci and enterobacteria groups, and reinforces the importance of *in vitro* susceptibility patterns of isolated pathogens to improve the success of therapy protocols.

This is the first report of septic arthritis in a large number of affected horses in Brazil, showing diversity of the etiology and multidrug resistance of the isolates.

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